



OIIPE

RAW SEQUENCE LISTING

DATE: 02/05/2002

PATENT APPLICATION: US/10/022,249

TIME: 14:19:13

Input Set : N:\jumbos\022249

Output Set: N:\CRF3\02052002\J022249.raw

ENTERED

4 <110> APPLICANT: Vega, Manuel
 5 Drittanti, Lila
 7 <120> TITLE OF INVENTION: HIGH THROUGHPUT DIRECTED EVOLUTION BY RATIONAL MUTAGENESIS
 9 <130> FILE REFERENCE: 37851-911
 C--> 11 <140> CURRENT APPLICATION NUMBER: US/10/022,249
 C--> 12 <141> CURRENT FILING DATE: 2001-12-17
 14 <150> PRIOR APPLICATION NUMBER: 60/315,382
 15 <151> PRIOR FILING DATE: 2001-08-27
 17 <160> NUMBER OF SEQ ID NOS: 735
 19 <170> SOFTWARE: FastSEQ for Windows Version 4.0
 21 <210> SEQ ID NO: 1
 22 <211> LENGTH: 621
 23 <212> TYPE: PRT
 24 <213> ORGANISM: Artificial Sequence
 26 <220> FEATURE:
 27 <223> OTHER INFORMATION: Mutant rep protein: rep78 4 GCT
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 31 1 5 10 15
 32 Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Val Ala Glu
 33 20 25 30
 34 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
 35 35 40 45
 36 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
 37 50 55 60
 38 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 39 65 70 75 80
 40 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
 41 85 90 95
 42 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
 43 100 105 110
 44 Arg Glu Lys Leu Ile Gln Arg Ile Tyr Arg Gly Ile Glu Pro Thr Leu
 45 115 120 125
 46 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
 47 130 135 140
 48 Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
 49 145 150 155 160
 50 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Gln Tyr Leu
 51 165 170 175
 52 Ser Ala Cys Leu Asn Leu Thr Glu Arg Lys Arg Leu Val Ala Gln His
 53 180 185 190
 54 Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn
 55 195 200 205

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56 Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
57      210      215      220
58 Met Glu Leu Val Gly Trp Leu Val Asp Lys Gly Ile Thr Ser Glu Lys
59 225      230      235      240
60 Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
61      245      250      255
62 Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
63      260      265      270
64 Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Gln
65      275      280      285
66 Pro Val Glu Asp Ile Ser Ser Asn Arg Ile Tyr Lys Ile Leu Glu Leu
67      290      295      300
68 Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala
69 305      310      315      320
70 Thr Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
71      325      330      335
72 Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
73      340      345      350
74 Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
75      355      360      365
76 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
77      370      375      380
78 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
79 385      390      395      400
80 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
81      405      410      415
82 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
83      420      425      430
84 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
85      435      440      445
86 Glu Leu Thr Arg Arg Leu Asp His Asp Phe Gly Lys Val Thr Lys Gln
87      450      455      460
88 Glu Val Lys Asp Phe Phe Arg Trp Ala Lys Asp His Val Val Glu Val
89 465      470      475      480
90 Glu His Glu Phe Tyr Val Lys Lys Gly Gly Ala Lys Lys Arg Pro Ala
91      485      490      495
92 Pro Ser Asp Ala Asp Ile Ser Glu Pro Lys Arg Val Arg Glu Ser Val
93      500      505      510
94 Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Ser Ile Asn Tyr Ala Asp
95      515      520      525
96 Arg Tyr Gln Asn Lys Cys Ser Arg His Val Gly Met Asn Leu Met Leu
97      530      535      540
98 Phe Pro Cys Arg Gln Cys Glu Arg Met Asn Gln Asn Ser Asn Ile Cys
99 545      550      555      560
100 Phe Thr His Gly Gln Lys Asp Cys Leu Glu Cys Phe Pro Val Ser Glu
101      565      570      575
102 Ser Gln Pro Val Ser Val Val Lys Lys Ala Tyr Gln Lys Leu Cys Tyr
103      580      585      590
104 Ile His His Ile Met Gly Lys Val Pro Asp Ala Cys Thr Ala Cys Asp

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106 Leu Val Asn Val Asp Leu Asp Asp Cys Ile Phe Glu Gln
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111 <212> TYPE: PRT
112 <213> ORGANISM: Artificial Sequence
114 <220> FEATURE:
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117 <400> SEQUENCE: 2
118 Thr Ala Gly Ala Tyr Glu Ile Val Ile Lys Val Pro Ser Asp Leu Asp
119 1          5          10          15
120 Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Val Ala Glu
121          20          25          30
122 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
123          35          40          45
124 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
125          50          55          60
126 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
127 65          70          75          80
128 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
129          85          90          95
130 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
131          100          105          110
132 Arg Glu Lys Leu Ile Gln Arg Ile Tyr Arg Gly Ile Glu Pro Thr Leu
133          115          120          125
134 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
135          130          135          140
136 Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
137 145          150          155          160
138 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Gln Tyr Leu
139          165          170          175
140 Ser Ala Cys Leu Asn Leu Thr Glu Arg Lys Arg Leu Val Ala Gln His
141          180          185          190
142 Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn
143          195          200          205
144 Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
145          210          215          220
146 Met Glu Leu Val Gly Trp Leu Val Asp Lys Gly Ile Thr Ser Glu Lys
147 225          230          235          240
148 Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
149          245          250          255
150 Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
151          260          265          270
152 Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Gln
153          275          280          285
154 Pro Val Glu Asp Ile Ser Ser Asn Arg Ile Tyr Lys Ile Leu Glu Leu
155          290          295          300
156 Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala

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```

157 305          310          315          320
158 Thr Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
159          325          330          335
160 Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
161          340          345          350
162 Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
163          355          360          365
164 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
165          370          375          380
166 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
167 385          390          395          400
168 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
169          405          410          415
170 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
171          420          425          430
172 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
173          435          440          445
174 Glu Leu Thr Arg Arg Leu Asp His Asp Phe Gly Lys Val Thr Lys Gln
175          450          455          460
176 Glu Val Lys Asp Phe Phe Arg Trp Ala Lys Asp His Val Val Glu Val
177 465          470          475          480
178 Glu His Glu Phe Tyr Val Lys Lys Gly Gly Ala Lys Lys Arg Pro Ala
179          485          490          495
180 Pro Ser Asp Ala Asp Ile Ser Glu Pro Lys Arg Val Arg Glu Ser Val
181          500          505          510
182 Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Ser Ile Asn Tyr Ala Asp
183          515          520          525
184 Arg Leu Ala Arg Gly His Ser Leu
185          530          535
187 <210> SEQ ID NO: 3
188 <211> LENGTH: 621
189 <212> TYPE: PRT
190 <213> ORGANISM: Artificial Sequence
192 <220> FEATURE:
193 <223> OTHER INFORMATION: Mutant rep protein: rep78 10 GCG
195 <400> SEQUENCE: 3
196 Thr Ala Gly Phe Tyr Glu Ile Val Ile Ala Val Pro Ser Asp Leu Asp
197 1          5          10          15
198 Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Val Ala Glu
199          20          25          30
200 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
201          35          40          45
202 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
203          50          55          60
204 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
205 65          70          75          80
206 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
207          85          90          95
208 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile

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| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 209 | | | | 100 | | | | 105 | | | | 110 | | | | |
| 210 | Arg | Glu | Lys | Leu | Ile | Gln | Arg | Ile | Tyr | Arg | Gly | Ile | Glu | Pro | Thr | Leu |
| 211 | | | 115 | | | | | 120 | | | | 125 | | | | |
| 212 | Pro | Asn | Trp | Phe | Ala | Val | Thr | Lys | Thr | Arg | Asn | Gly | Ala | Gly | Gly | Gly |
| 213 | | | 130 | | | | | 135 | | | | 140 | | | | |
| 214 | Asn | Lys | Val | Val | Asp | Glu | Cys | Tyr | Ile | Pro | Asn | Tyr | Leu | Leu | Pro | Lys |
| 215 | 145 | | | | | 150 | | | | 155 | | | | | | 160 |
| 216 | Thr | Gln | Pro | Glu | Leu | Gln | Trp | Ala | Trp | Thr | Asn | Met | Glu | Gln | Tyr | Leu |
| 217 | | | | | 165 | | | | | 170 | | | | | 175 | |
| 218 | Ser | Ala | Cys | Leu | Asn | Leu | Thr | Glu | Arg | Lys | Arg | Leu | Val | Ala | Gln | His |
| 219 | | | | 180 | | | | | 185 | | | | | 190 | | |
| 220 | Leu | Thr | His | Val | Ser | Gln | Thr | Gln | Glu | Gln | Asn | Lys | Glu | Asn | Gln | Asn |
| 221 | | | 195 | | | | | 200 | | | | | 205 | | | |
| 222 | Pro | Asn | Ser | Asp | Ala | Pro | Val | Ile | Arg | Ser | Lys | Thr | Ser | Ala | Arg | Tyr |
| 223 | | 210 | | | | | | 215 | | | | 220 | | | | |
| 224 | Met | Glu | Leu | Val | Gly | Trp | Leu | Val | Asp | Lys | Gly | Ile | Thr | Ser | Glu | Lys |
| 225 | 225 | | | | | 230 | | | | 235 | | | | | | 240 |
| 226 | Gln | Trp | Ile | Gln | Glu | Asp | Gln | Ala | Ser | Tyr | Ile | Ser | Phe | Asn | Ala | Ala |
| 227 | | | | | 245 | | | | | 250 | | | | | 255 | |
| 228 | Ser | Asn | Ser | Arg | Ser | Gln | Ile | Lys | Ala | Ala | Leu | Asp | Asn | Ala | Gly | Lys |
| 229 | | | | 260 | | | | | 265 | | | | | 270 | | |
| 230 | Ile | Met | Ser | Leu | Thr | Lys | Thr | Ala | Pro | Asp | Tyr | Leu | Val | Gly | Gln | Gln |
| 231 | | 275 | | | | | | 280 | | | | | 285 | | | |
| 232 | Pro | Val | Glu | Asp | Ile | Ser | Ser | Asn | Arg | Ile | Tyr | Lys | Ile | Leu | Glu | Leu |
| 233 | | 290 | | | | | | 295 | | | | 300 | | | | |
| 234 | Asn | Gly | Tyr | Asp | Pro | Gln | Tyr | Ala | Ala | Ser | Val | Phe | Leu | Gly | Trp | Ala |
| 235 | 305 | | | | | 310 | | | | 315 | | | | | | 320 |
| 236 | Thr | Lys | Lys | Phe | Gly | Lys | Arg | Asn | Thr | Ile | Trp | Leu | Phe | Gly | Pro | Ala |
| 237 | | | | | 325 | | | | | 330 | | | | | 335 | |
| 238 | Thr | Thr | Gly | Lys | Thr | Asn | Ile | Ala | Glu | Ala | Ile | Ala | His | Thr | Val | Pro |
| 239 | | | | 340 | | | | | 345 | | | | | 350 | | |
| 240 | Phe | Tyr | Gly | Cys | Val | Asn | Trp | Thr | Asn | Glu | Asn | Phe | Pro | Phe | Asn | Asp |
| 241 | | | 355 | | | | | 360 | | | | | 365 | | | |
| 242 | Cys | Val | Asp | Lys | Met | Val | Ile | Trp | Trp | Glu | Glu | Gly | Lys | Met | Thr | Ala |
| 243 | | 370 | | | | | | 375 | | | | 380 | | | | |
| 244 | Lys | Val | Val | Glu | Ser | Ala | Lys | Ala | Ile | Leu | Gly | Gly | Ser | Lys | Val | Arg |
| 245 | 385 | | | | | 390 | | | | 395 | | | | | | 400 |
| 246 | Val | Asp | Gln | Lys | Cys | Lys | Ser | Ser | Ala | Gln | Ile | Asp | Pro | Thr | Pro | Val |
| 247 | | | | | 405 | | | | | 410 | | | | | 415 | |
| 248 | Ile | Val | Thr | Ser | Asn | Thr | Asn | Met | Cys | Ala | Val | Ile | Asp | Gly | Asn | Ser |
| 249 | | | | 420 | | | | | 425 | | | | | 430 | | |
| 250 | Thr | Thr | Phe | Glu | His | Gln | Gln | Pro | Leu | Gln | Asp | Arg | Met | Phe | Lys | Phe |
| 251 | | | 435 | | | | | 440 | | | | | 445 | | | |
| 252 | Glu | Leu | Thr | Arg | Arg | Leu | Asp | His | Asp | Phe | Gly | Lys | Val | Thr | Lys | Gln |
| 253 | | 450 | | | | | 455 | | | | | 460 | | | | |
| 254 | Glu | Val | Lys | Asp | Phe | Phe | Arg | Trp | Ala | Lys | Asp | His | Val | Val | Glu | Val |
| 255 | 465 | | | | | 470 | | | | 475 | | | | | | 480 |
| 256 | Glu | His | Glu | Phe | Tyr | Val | Lys | Lys | Gly | Gly | Ala | Lys | Lys | Arg | Pro | Ala |
| 257 | | | | | 485 | | | | | 490 | | | | | 495 | |

VERIFICATION SUMMARY

PATENT APPLICATION: US/10/022,249

DATE: 02/05/2002

TIME: 14:19:14

Input Set : N:\jumbos\022249

Output Set: N:\CRF3\02052002\J022249.raw

L:11 M:270 C: Current Application Number differs, Replaced Current Application Number
L:12 M:271 C: Current Filing Date differs, Replaced Current Filing Date